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ROLE OF TRACE ELEMENTS IN NUTRITION

By W. D. McELROY, Ph.D. and ALVIN NASON, Ph.D.*

INTRODUCTION

All forms of life require a number of inorganic elements for normal growth and reproduction. While virtually all the elements of the periodic table have been found in living cells, not all of these are essential to life. When an element is required in relatively high concentrations in order to maintain normal growth and reproduction of the organisms, it is not usually difficult to devise a diet which will show its essentiality. In this class are such nutrients as sulfur, phosphorus, magnesium, calcium and nitrogen. In addition, for animals, sodium and chloride ions must be added to the list of major or *macronutrient elements.* In contrast to this group of elements there are those which are required in very small amounts, the *micronutrients* or "trace elements." Although the need for the trace elements has been established by rigidly controlled experimental procedures, in most cases the first clues to their nutritional importance were indicated by deficiency diseases. A notable example is the case of cobalt. Various diseases of sheep and cattle have been traced to a deficiency of this element. Morton's Main disease in Australia and New Zealand, bush disease in Europe, salt-lick disease in Florida are apparently related forms of anemia which respond to cobalt given directly, or indirectly by applying fertilizers to the soil (1). Cobalt has since been shown to be the metal component of the antipernicious anemia vitamin B_{12} (2). Cobalt, as an element, has been shown to be important only in the diets of ruminants. Even in this case it is the microorganisms in the rumen which utilize it for the synthesis of vitamin B_{12} (2a). The essentiality of vitamin B_{12} for the prevention of certain anemias is used as an argument for the essentiality of cobalt for all animals. The argument is a good one but not conclusive. It may be that the organic moiety of vitamin B_{12} is the essential part for function, while cobalt may merely serve in directing its synthesis in the microorganism or by stabilizing the structure once formed. Until a specific, essential function or general need for cobalt is found it cannot be stated with certainty that it is required for all higher animals. There are other

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interesting examples among the elements which cause us to reflect on the definition of essentiality. It seems clear that fluoride ion prevents dental caries when used in appropriate concentration. However, there is no nutritional evidence to suggest that it is essential for normal growth and reproduction of the organism as a whole. Further study is necessary in order to clarify the role of certain metals in nutrition. Under some conditions even "non-essential" elements may have a beneficial effect.

The absolute nutritional requirement and mechanism of action of metals in enzymatic processes has clearly established the essentiality of a number of these elements. Iron, copper, zinc, manganese, iodine, and probably cobalt are considered the essential trace metals for animals. Molybdenum has been shown to be important in certain animal enzyme systems. However, a molybdenum deficiency related to inadequate growth has not been observed. Although all workers are willing to accept manganese as an essential element, a clear cut manganese deficiency syndrome for higher animals is not easily obtained. In rats manganese deficiency has been shown to affect reproduction while in birds a deficiency brings about a disease of young chicks known as perosis or slipped tendon. The disease is characterized by shortened leg and wing bones and particularly by the malformation of the tibio-metatarsal joint which results in the displacement of the tendon. Specific diseases related to other trace metal deficiencies such as the anemias caused by copper and iron deficiencies, and goiter and cretinism due to iodine deficiency have been extensively discussed by various authors (see Monier-Williams (3) and Follis (4).)

Source of Trace Elements

The mineral elements are obtained primarily from the soil and water. Therefore all animals ultimately depend upon plants and water for their mineral requirements. It is important to recognize, however, that there are some critical differences in the requirements of animals and plants. For example, as far as we know plants do not require iodine for normal development. For this reason plants grown around the Great Lakes area and in the Pacific Northwest (the two large goiter areas) are deficient in iodine. Fortunately, the distribution of iodine through salt has done much to reduce this nutritional hazard. It is interesting that plants do accumulate iodine from the soil or water if it is present. Seaweeds and plankton on which fish feed are particularly rich in this element. The other trace metals which were discussed as essential for animals have all been shown to be essential for plants. Using both nutritional and enzymatic criteria, molybdenum has been shown to be essential for nitrogen metabolism. Boron and vanadium are essential for plants but thus far a requirement for animals has not been established.

Vanadium is accumulated by Ascidians and it has been suggested that this element may replace iron or copper in the respiratory pigments of these animals.

In general one might conclude that trace metal deficiency in man in the United States would be rare since in most instances the diets are composed of foods which are cultivated or raised in various parts of the country. However, this does not preclude the possibility that there may be individual differences relative to absolute requirements. Adsorption, excretion and even chelating agents (drugs) in the diet may materially affect the available trace metal supply. In addition, serious objections can be raised against using growth as the sole criterion of nutritional adequacy.

Catalytic Role of Trace Metals

Our knowledge of the role of trace elements in nutrition has expanded rapidly during the last two decades, due primarily to research on specific enzyme systems from animals, plants and microorganisms. The fact that trace elements are needed in only small quantities is taken as evidence that they are functioning in some catalytic role. In this respect the function of trace elements is similar to that of the organic micronutrients — the vitamins. Except for iodine which has been shown to be essential as a component of the thyroglobulin molecule and cobalt which is part of the vitamin B_{12} molecule, a known enzymatic function has been described for the trace metals using highly purified preparations.

In many cases the free metals show certain inherent catalytic properties which are remarkably enhanced and made more specific when bound to particular proteins. The copper ion catalysis of the oxidation of ascorbic acid is increased at least 1000-fold by the copper-containing enzyme, ascorbic acid oxidase. Another major difference in this case is that no hydrogen peroxide is produced by the enzymatic reaction, indicating a possible change in the actual mechanism.

One finds less oxidase and electron transport activity in the inorganic iron salts than in the iron containing heme proteins. The important question concerning the function of trace metals in enzyme-catalyzed reactions is that of their mechanism of action in increasing catalysis after combining with protein. There have been a number of excellent reviews (5-11) concerning the properties of the ions and the structural linkages between metals and organic molecules. Many amino acids form metal complexes by coordination through the carboxyl and amino groups. In the case of protein molecules, however, only certain specific polar side chains act as linkages for the formation of protein complexes with metals. These are the carboxyl, imidazolium, phenolic and sulfhydryl groups.

Klotz (7) emphasized three general categories in which metals in combination with proteins or prosthetic groups may act as catalysts: (a) primary effect on the properties of metals, (b) primary effect on the characteristics of the enzyme protein, and (c) cooperative effects of metal and protein. The best known examples of the first group are enzymes which participate in oxidation-reduction reactions, such as some of the iron and copper enzymes cited above.

In the second category, the combination of metals with protein may lead to an alteration of a number of properties of the latter. The combination will alter the net charge of the protein and from purely electrostatic effects may possibly alter the combination of substrates with enzymes. As an illustration, one can cite the work of Sadasivan (12) indicating a shift in the pH optimum of phosphatase either to the alkaline or acid range according to changes in the ratio of zinc and magnesium. If the dissociation of groups essential for enzymatic activity is altered by the presence of metals, it is to be expected that metal activation or depression will vary with pH. The electrostatic effects of metals on proteins may be responsible for the profound influence of non-essential metals on the metabolism of both animals and plants. In the third category the predominant idea is that metals and proteins may act cooperatively to increase catalytic activity. Hellerman and Stock (13) were among the first to suggest that metals may serve as a bridge between substrate and protein. Smith *et al.* (8) has presented additional evidence for the formation of a chelate structure between metal ion and the substrate as a prerequisite for enzymatic activity. In the case of the manganese-requiring enzyme prolidase, a dipeptidase which catalyzes the hydrolysis of glycyl-l-proline, he has proposed a five-member ring structure for the manganese-substrate complex. On the other hand Klotz (7) has proposed an alternate mechanism for this type of reaction. He suggests that the primary function of the metal is to favor the formation of an active intermediate in which the products are actively dissociated from the metal protein.

The ability of an ion to combine with a substrate and with an enzyme is not sufficient to produce biological activity. In the case of enolase, the enzyme catalyzing the conversion of 2-phosphoglycerate to phosphoenol pyruvate, Malmstrom (14) has shown that beryllium, calcium and nickel, which form complexes with the enzyme, actually inhibit activity. He demonstrated that although magnesium ions form the weakest complex with enolase and substrate, they give the best biological action. Zinc on the other hand is strongly bound and also shows relatively high activity. It is thus apparent that the effect of metal ions in activating enzymes must have some specificity beyond mere combination with the substrate and protein.

Metal Requirements of Enzymes

Although the present discussion is directed primarily to the trace elements, other metals such as magnesium, potassium and sodium have been included when they have been shown to influence the catalytic properties of a particular protein. In terms of the metal requirements of enzymes, two broad groups can be drawn up: (1) those enzymes for which one or more metals serve as an activator and (2) those enzymes in which a specific metal has been shown to be an integral component.

Enzymes and Metal Ion Activators

Magnesium plays a predominant role in the activity of the various enzymes concerned with glycolysis and the early stages of carbohydrate metabolism. In most cases manganese will substitute for magnesium, but its activity is somewhat lower. It is of interest that in 1931 McCollum first described the spectacular syndrome of magnesium deficiency in rats and dogs consisting of convulsions and hyperemia. In the same year Lohmann demonstrated the importance of magnesium in muscle glycolysis. Of the variety of sugar phosphorylating enzymes which have now been studied in detail, all require magnesium as the activating metal. That magnesium is essential for phosphate-transferring enzymes in general is indicated by the fact that virtually all the kinases including myokinase (15), diphosphocoenzyme A kinase (16), diphosphopyridine nucleotide kinase (17), and creatine kinase (18) require magnesium in the enzymatic transfer of the phosphate group from adenosine triphosphate to the appropriate substrate. It seems clear that magnesium, and to a certain extent manganese, are required primarily for those reactions involving group transfer. This is particularly true of those reactions in which a pyrophosphate linkage is involved. The presence of a pyrophosphate structure in many of the cofactors and substrates involved in group transfer suggests that a chelate structure with magnesium or manganese is probable. It is of some nutritional interest that manganese has been implicated in the conversion of monosaccharides to ascorbic acid (19). This could have particular relevance for animals which do not require vitamin C in their diet.

A number of metals have been shown to be important in activating the enzymes associated with the tricarboxylic acid cycle. Stadtman (20) has shown stimulation of phosphotransacetylase by potassium ions, and Von Korff (21) has demonstrated a marked stimulation of the acetate activating enzyme by the same metal. Ammonium or rubidium ions will also function in this manner, while sodium ions act as strong inhibitors. Manganese has been shown to be essential for the enzyme which catalyzes the conversion of

isocitric acid to ketoglutaric acid. Ochoa and coworkers (22-23) have implicated manganese in the second step of this reaction, namely in the enzymatic decarboxylation of oxalosuccinic acid to ketoglutarate rather than in the preceding dehydrogenation of isocitrate to oxalosuccinate. This is in keeping with the requirement of manganese for the enzymatic decarboxylation of oxaloacetic acid to pyruvate (24). Other metal-requiring enzymes of the Krebs cycle include pyruvic carboxylase, pyruvic oxidase and the condensing enzyme. All of these are activated by magnesium or manganese ions and in some cases by other ions such as AL^{+++}, Co^{++} or Zn^{++}. It appears then that magnesium ions play an important role in the glycolytic cycle in which phosphate transfer is essential, whereas manganese is the predominant ion in the Krebs cycle. This effect of Mn^{++} is related to the important oxidative and non-oxidative decarboxylation steps which occur during the metabolism of di- and tricarboxylic acids.

It is clear from the example cited above that certain enzymes may be activated by a number of metals. Some additional examples in which a variety of metals will serve as activators include the enzymes arginase, lecithinase, desoxyribonuclease, and histidine deaminase.

Specific Metal Components of Enzymes

A number of the trace elements have been clearly established as specific and integral components of particular enzymes in much the same manner as are some of the vitamins. Table I lists some of these highly specific metalloenzymes.

Zinc has been shown to be a constituent of the enzyme carbonic anhydrase (25) which catalyzes the combination of carbon dioxide and water in tissues and blood to form carbonic acid. Zinc thus plays an important role in the respiratory system, and for this reason it is essential to include in the diet of animals at least 0.004 grams of zinc per day. More recently zinc has been implicated as the specific metal component of carboxypeptidase (26) and of a number of pyridine nucleotide dehydrogenases. The latter include yeast and liver alcohol dehydrogenases (27-29), liver glutamic dehydrogenase (30) and liver lactic dehydrogenase (31). It has also been reported that the amount of zinc in food stuffs is positively correlated with that of thiamine, which suggests that zinc deficiency may be a factor in the polyneuritis syndrome (32, 33).

A number of functions for iron have been established. A deficiency of iron produces anemia in animals, for 66 per cent of the iron in the body is contained in the hemoglobin of the blood, the remainder being located primarily at those sites where red blood cells are formed. Cobalt in the form of

TABLE I

Specific Metallo Enzymes

Enzyme	Reaction	Metal
Carbonic anhydrase	$CO_2 + H_2O \rightleftarrows H_2CO_3$	Zn
Dehydropeptidase	Glycyldehydrophenylanine $\rightarrow NH_3$ + penylpyruvic A	Zn
Glycylglycine dipeptidase	Glycylglycine $\rightarrow$ glycine	Zn
Carboxypeptidase	Carbobenzoxyglycyl-L-phenylalanine $\rightarrow$ phenylalanine	Zn
Alcohol dehydrogenase	Ethanol + DPN $\rightleftarrows$ acetaldehyde + DPNH	Zn
Glutamic dehydrogenase	Glutamate + DPN $\rightleftarrows$ ketoglutarate + DPNH + NH_3	Zn
Lactic dehydrogenase	Lactate + DPN $\rightleftarrows$ pyruvate + DPNH	Zn
Inorganic pyrophosphatase	Pyrophosphate + $H_2O \leftarrow PO_4$	Mg
Fumaric hydrogenase	Fumaric acid + 2H $\rightarrow$ succinic acid	Fe
Catalase	$2H_2O_2 \rightarrow 2H_2O + O_2$	Fe
Peroxidase	H_2O_2 oxidation of aromatic amines and other compounds	Fe
Cytochromes	Electron transport	Fe
DPNH-cytochrome c reductase	DPNH + cytochrome c (Fe+++) $\rightarrow$ DPN + cytochrome c (Fe++)	Fe
Uricase	Uric acid + $O_2 \rightleftarrows$ allantoin + H_2O_2 + CO_2	Cu
Tyrosinase	Tyrosine + $\frac{1}{2}O_2 \rightarrow$ hallochrome	Cu
Laccase	Phenols $\rightarrow$ ortho and para quinones	Cu
Ascorbic acid oxidase	Ascorbic acid $\rightarrow$ dehydroascorbic A	Cu
Butyryl CoA dehydrogenase	Butyryl CoA-2e $\rightarrow$ crotonyl-CoA	Cu
Prolidase	Glycylproline $\rightarrow$ proline	Mn
Nitrate reductase	NO_3 + TPNH + H+ $\rightarrow NO_2$ + TPN+ + H_2O	Mo
Xanthine oxidase	Xanthine + $O_2 \rightarrow H_2O$ + uric acid	Mo
Aldehyde oxidase	Acetaldehyde + $O_2 \rightarrow$ acetate + H_2O	Mo

vitamin B_{12} and copper also are intimately associated with the formation of hemoglobin, for anemic patients who do not respond to iron dosage often do respond when vitamin B_{12} or copper or both are added to the diet. Unfortunately very little can be said concerning the role of these metals in hemoglobin and red blood cell synthesis. Iron in living cells is found chiefly in the form of porphyrins. As indicated above it appears for the most part in vertebrates as the hemoglobin of the erythrocytes. The erythrocruorins are the corresponding iron-containing respiratory pigments in the blood and tissue fluids of some invertebrates; and the myoglobins are the iron-containing respiratory pigments occurring in muscle cells of vertebrates and invertebrates. At the enzyme level, the peroxidases and catalases (34) are iron-porphyrin-containing enzymes that catalyze reactions in which hydrogen peroxide is an electron acceptor. The terminal respiratory chain in animals, which involves the transfer of electrons from the substrate level (e.g., reduced pyridine nucleotides, succinate) to oxygen are mediated almost exclusively by the iron-porphyrin containing series of cytochromes including cytochromes b, b_5, c_1,

c, a, and a_3 (34). The iron of the cytochromes undergoes alternate oxidation-reduction between Fe^{+++} and Fe^{++} during electron transport. The terminal respiratory chain is of particular importance since much of the useful energy of foodstuffs is released by these oxidative steps. One of the very active fields in biochemistry today is concerned with the mechanism of release and conservation of energy during electron transport by these metal catalysts. Copper is also associated with these electron transport systems and is known to be involved in fatty acid oxidation (35).

In addition, copper has been shown conclusively to be the specific metal component of the enzymes tyrosinase, laccase and ascorbic acid oxidase (36). Tyrosinase (or polyphenol oxidase), in conjunction with accompanying monophenolase activity, catalyzes the conversion of the essential amino acid tyrosine to the orthoquinone form, dihydroxyphenylalanine quinone, which is then further converted to melanin pigments. Laccase, found for the most part in certain plants, mediates the oxidation of para-hydroxy aryl compounds, and ascorbic acid oxidase accelerates the conversion of ascorbic acid to dehydroascorbic acid. The mechanism of action of the copper in polyphenol oxidase has been demonstrated by Kubowitz (37) to be due to its electron transport role, undergoing cyclic oxidation-reduction between Cu^{++} and Cu^{+} during the enzymatic transfer of electrons from substrate to oxygen. The evidence (38) strongly suggests that the copper of ascorbic acid oxidase acts in a similar fashion. Aside from the importance of tyrosinase in the normal pigmentation of animals, pigmentation in melanoma tumors has been correlated with presence of tyrosinase by Hogeboom and Adams (39). The copper enzymes also appear to have a significant role in terminal respiration of plants. Recently Mahler and coworkers (40) have reported that purified samples of kidney uricase contain 0.05 per cent copper.

Molybdenum concentration in the diet has been shown to affect the activity of tissue xanthine oxidase, a flavo-protein (41, 42). The enzyme contains molybdenum as one of its components (43-37). In addition it has also been reported that liver aldehyde oxidase is a molybdoflavoprotein, the metal being required for the reduction of cytochrome c but not dyes or oxygen (48). It is very likely that the molybdenum in these enzymes is acting in an electron transport role, undergoing alternation oxidation-reduction as has been demonstrated for the molybdoflavoprotein nitrate reductase from the fungus *Neurospora* (49).

In recent years it has become fashionable to assume that a metal is essential if it is found to be required specifically for the function of an enzyme. In this case, however, one must assume that the enzyme itself is essential. In some cases, as with the iron porphyrin enzymes, this is a reasonable assump-

tion. In other cases the evidence is not so clear. Is xanthine oxidase essential for growth and reproduction? If it is and molybdenum is required for its synthesis or function, then molybdenum can be classified as an essential element. Our present knowledge concerning the induced formation of enzymes suggests that some of these catalysts can be eliminated under certain nutritional conditions without affecting the viability of the organism. Thus, what may be an essential element with one diet may not be for a second diet. The adaptation of microorganisms to use certain substrates for growth is an excellent example. Also the minimal requirement for metals varies considerably, depending upon the diet. Adsorption and excretion are important in this respect. High calcium and phosphate raises the requirements for manganese. Similar relationships are noted for other metals. A molybdenum deficiency is easily obtained in plants grown on nitrate as the sole nitrogen source while with ammonia as the nitrogen source such a deficiency is rarely observed. Molybdenum is known to be required for the function of the enzyme nitrate reductase.

Trace Element Nutrition and Enzyme Patterns

With the findings that metal ions are vitally concerned with the action of numerous enzymes, it is not surprising that when animals, plants or microorganisms are provided inadequate or excessive supplies of the minerals, alterations in the enzymatic constitution of the tissues occur. Also, the necessity of a metal ion for the activity or synthesis of specific enzymes may be revealed by growing the organisms under conditions of metal deficiency and comparing their enzyme systems with those of normal tissues. Extensive studies on the concentration of various enzymes in tissues as affected by metal deficiencies or toxicities have brought to light some interesting aspects of trace element function. It has been shown, for example, that a deficiency of zinc or biotin in the fungus *Neurospora* does not lead only to the production of less mycelium but actually results in the production of tissue with drastically altered metabolic characteristics, as indicated by the marked changes in enzymatic constitution (50). The alterations involve not only the virtual disappearance of the activity of such enzymes as alcohol dehydrogenase and tryptophane synthetase, but also remarkable increases in the activity of other enzymes. An example of the latter is the 10- to 20-fold rise in the concentration of diphosphopyridine nucleotidase (DPNase). On the other hand, certain enzymes including fumarase, hexokinase, aldolase, and triosephosphate dehydrogenase were unaffected. In studies on the role of micronutrients in the metabolism of higher plants it was shown that individual trace element deficiencies caused

an alteration in the over-all oxidative enzyme pattern which was characteristic of the micronutrient element in question (51). It has also been demonstrated that the alkaline phosphatase and diphosphopyridine nucleotidase activities of the brain in ducklings with magnesium deficiency were significantly lower than for the controls, whereas inorganic pyrophosphatase and cytochrome oxidase activities were not affected (52). The alkaline phosphatase activity of liver, kidney, heart and plasma of manganese-deficient ducklings was also decreased when compared with the controls (53). The mechanism by which trace element deficiencies lead to an alteration of enzyme pattern is not entirely clear. In the case of a specific metallo-enzyme it is not surprising that a deficiency of the particular metal leads to a decreased concentration of that enzyme. The obvious explanation would be the failure of incorporation of the necessary element into the enzyme, or failure to stimulate synthesis of the protein portion. On the other hand, in the case of increased activities of certain enzymes, one can only speculate that these are primitive proteins of relatively simple structure, the synthesis of which can proceed even in the absence of certain reactions which are necessary for the binding of more complex protein molecules. Regardless of the explanation, it is important to recognize that a single metal deficiency does not lead just to a reduction of enzymatic activity. The increased activity of other systems may be of considerable significance in understanding the pathology of a particular disease.

In studies of the effect of excessive dietary zinc on various enzymes in the liver of rats, Van Reen (54) observed that the iron-containing catalase and cytochrome oxidase were reduced 50 to 70 per cent. There was also a simultaneous reduction in hemoglobin. The report by Smith and Larson (55) showing the anemia produced by feeding zinc salts can be overcome by supplementing the diet with 0.2 mg. copper per day prompted an examination of the above enzymes. The administration of copper sulfate to the animals fed the zinc-toxic diet resulted in liver catalase and cytochrome oxidase activities that were considerably higher than in the unsupplemented toxic animals (54). The anemia produced by excessive zinc was also corrected by copper supplementations. The observations are consistent with the view that excessive zinc interferes with copper metabolism which is manifested in a reduction of the iron proteins such as hemoglobin, catalase and cytochrome oxidase. The results are of particular interest since the recent findings that zinc is important in electron transport systems.

The report of Ferguson *et al.* (56) that the severe symptoms in cattle and sheep resulting from molybdenum toxicity could be corrected by copper sulfate administration prompted an examination of the enzyme pattern changes.

Using rats Van Reen (57) could show that molybdenum toxicity resulted in a 9-fold increase in the liver alkaline phosphatase, whereas catalase, cytochrome oxidase, isocitric dehydrogenase and hemoglobin values were not severely affected. Alkaline phosphatase of kidney and heart, however, were unaffected. From the above enzymatic studies it would appear that the abnormalities due to the influence of excessive dietary molybdenum are not a result of altered copper metabolism. It is of interest that the above enzymatic response of the liver has proven to be a sensitive indicator of molybdenum toxicity, for at levels of molybdenum where growth and general appearance were normal, increased liver alkaline phosphatase was still observed.

Grey and Daniel (58) reported that copper plus methionine were effective in overcoming the growth-depressing action of molybdenum in rats. Subsequently Van Reen found that both copper and methionine were also effective in correcting the enzyme changes due to molybdenum toxicity. From recent studies by Dick (59) in Australia on molybdenum toxicity in sheep, and by Van Reen and coworkers in this country, it appears that the reason for the effectiveness of methionine is due to the formation of sulfate from the sulfur. Following these leads it has now been shown (60) that inorganic sulfate will also lower the high level of liver alkaline phosphatase activity. An explanation for these interesting relationships is still lacking. The fact that sulfate is very specific in reversing the toxicity suggests the possibility that those reactions concerned with sulfate formation may be affected by excess molybdenum.

The earlier studies showing a relationship between copper and molybdenum appear to be explained on the basis that copper sulfate was used in the diets. In nutritional studies on trace metal deficiencies and toxicities, it is evident that both the cations and anions cannot be changed simultaneously.

Concluding Remarks

From the physico-chemical and nutritional studies on the influence and mechanism of action of metals in enzyme systems, several general patterns evolve. Although there are exceptions to these generalizations, it appears that those metals most closely associated with electron transferring systems are copper, iron and molybdenum. It is interesting that these metals have the inherent capacity to function as electron mediators in non-enzymatic oxidation-reduction reactions. The important biochemical questions are: (a) why, in combination with specific proteins, is this capacity for catalyzing oxidation-reduction reactions accentuated, and (b) what is the nature of the linkages which allow specific coupling of these metal systems to others, allowing the transfer of electrons along specific pathways? The pattern which seems to be

emerging is that these metals are not required specifically for the combination of substrate to the catalytic protein, but rather function primarily as "electron couplers" from one protein system to another.

It is clear from the examples cited that magnesium, and to a certain extent manganese, are required primarily for those reactions involving group transfer, in particular those in which phosphate participates. In recent years it has became increasingly clear that enzymes participate intimately in group transfer by serving as the intermediate carrier. Magnesium plays a predominant role promoting the formation of the enzyme-substrate complex and the resulting intermediate of the reaction. The presence of a pyrophosphate structure in many of the cofactors and substrates involved in group transfer suggests that a chelate structure with magnesium is probable.

The predominant metal involved in general enzymatic decarboxylation and hydrolysis reactions is manganese (and to a certain extent zinc and magnesium). At present there is no general agreement as to the primary mechanism of action of these metals. Certain workers feel that they form an essential structure with the substrate, thus acting to bring the substrate into combination with the protein. Other workers feel, however, that the metal combines with the enzyme and functions primarily to accelerate and therefore increase the concentration of an essential intermediate in the reaction. It is evident that manganese does not have strong inherent properties for catalyzing decarboxylation, whereas other metals such as copper, which do not function as cofactors in enzymatic decarboxylation, are very effective in nonenzymatic reactions. The suggestion, therefore, that manganese functions in enzyme systems by forming chelate structures with the substrate is lacking strong experimental support .

There is little question that a thorough knowledge of the specific phenomena related to the biochemical role of trace elements, *in vitro* and *in vivo*, may be of great value in interpreting nutritional disorders. From a diagnostic point of view, measurements of enzymatic activity, such as in the case of molybdenum toxicity cited above, may prove to be a very useful practical tool.

In recent years considerable attention has been given to the ability of organic molecules to form complexes with metals, i.e. chelates. A variety of drugs, used clinically at present, are powerful chelating agents. Many of the side effects produced by antibiotics may be due to the removel of certain metals. On the other hand, the antibacterial action of certain agents depends upon the presence of these trace elements. In several cases it has been suggested that the chelate structure is the biologically important compound. One of the more interesting examples of catalysis by a chelate structure is the oxygen transport characteristic of a cobalt-histidine chelate, reported to

increase the survival time of rats at high altitude (61). It is to be expected, therefore, that future studies on the nutritional significance of trace elements will reveal many new and interesting interrelationships. As emphasized earlier it has become increasingly clear that good health is not simply the absence of a disease. It is rather an active, dynamic state of well-being and we know that the trace metals are an integral part of the machinery which maintains this steady state.

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NUTRITION NOTES

A NUTRITIONAL PROGRAM FOR PROLONGATION OF LIFE IN CORONARY—L. M. MORRISON, *Atherosclerosis, J.A.M.A.,* 159:1425, 1955.

An interesting study is reported on 100 proved cases of myocardial infarction which were divided into two groups. One was given a diet restricted to 20 to 25 g of fat per day, while the other group was permitted to have an unrestricted "typical American diet." The low-fat diet consisted of approximately 1600 calories per day, derived as follows: 225 g of carbohydrate, 120 g of protein, and 50 to 75 ml of cholesterol per day. In addition, supplementary vitamin capsules were prescribed plus two ounces of whole-wheat germ and eight g of powdered brewer's yeast per day. After eight years of this study, 76% of the patients ingesting a non-restricted diet were dead compared to 44% of those who received the low-fat diet.

While such statistics are of significance, the problem of maintaining a patient on a markedly restricted diet containing only 20 to 25 g of fat per day is extremely difficult. Certainly in such a selected group as patients with myocardial infarction such a dietary regimen may be desirable. It should be pointed out, however, that such a diet should be followed under medical supervision, because in a sense it is markedly unbalanced. Additional data of this kind would be most valuable.

THE UNDERWEIGHT PATIENT AS AN INCREASED OBSTETRIC HAZARD — W. T. TOMPKINS, D. G. WIEHL, and R. MCN. MITCHELL, *Am. J. of Obs. and Gyne.,* 69:114, 1955.

The authors report that markedly underweight patients have a greater than average chance of toxemia and a greatly increased probability of premature labor. Patients who are 20% or more overweight or underweight have an increased probability of developing toxemia. The frequency of premature labor was more than twice as great in patients showing less than the average gain or a loss in weight in the first six months. In the case of the underweight patient, every effort must be made as early in pregnancy as possible to prevent the onset of spontaneous premature labor.

EVIDENCE FOR ABNORMAL VITAMIN B_6 METABOLISM IN PREGNANCY AND VARIOUS DISEASE STATES — M. WACHSTEIN, *The Amer. J. of Clin. Nutr.,* 4:369, 1956.

Evidence for a disturbed metabolism of vitamin B_6 in pregnancy is based on abnormalities of tryptophan metabolism. For example, in the absence of added B_6 there is a marked increase of xanthurenic acid excretion following a tryptophan load test. Furthermore, one observes an immediate return of tryptophan metabolism to normal following the administration of relatively small amounts of pyridoxin. In addition, an abnormal vitamin B_6 load test indicating a significantly larger retention of the administered vitamin in comparison to non-pregnant control subjects. The authors recommend the supplementation of a normal diet with 10 mg of pyridoxin hydrochloride daily in order to maintain a normal metabolism of tryptophan.

VITAMIN B_6 DEFICIENCY IN THE RHESUS MONKEY — WITH PARTICULAR REFERENCE TO THE OCCURRENCE OF ATHEROSCLEROSIS, DENTAL CARIES, AND HEPATIC CIRRHOSIS — JAMES F. RINEHART and LOUIS D. GREENBERG, *The Amer. J. of Clin. Nutr.,* 4:318, 1956.

This paper presents an interesting lead into the possible etiology of atherosclero-

sis. Rhesus monkeys were maintained on essentially synthetic diets, theoretically complete nutritionally with the exception of the vitamin pyridoxin. Pyridoxin-deficient animals regularly developed alterations in blood vessels with close similarities to atherosclerosis seen in man. In these experimental animals the vascular lesions developed after 5 to 6 months of complete deprivation of pyridoxin. In addition, pathological changes such as fatty infiltration and sclerosis of the liver were noted. It was further observed that monkeys maintained for two years or longer on a diet inadequate in pyridoxin developed a high incidence of dental caries. The authors raise the question as to whether or not the average daily intake of pyridoxin in man, which is 1.5 mg, is adequate to meet the metabolic needs. This interesting paper raises the question of whether or not this vitamin may play a role in the etiology of such important human diseases as atherosclerosis, dental caries, and cirrhosis of the liver.

DIETIC TREATMENT OF PEPTIC ULCER—R. DOLL, P. FRIEDLANDER, and F. PYGGOTT, *Lancet,* 1:5, 1956.

This work attempts to evaluate the traditional bland soft diet in the management of the peptic ulcer patient. In this study, 64 hospital patients were randomly divided into two groups. One was given the so-called hospital "ulcer" diet, while the other group was given an "almost normal" diet. Fried foods were omitted from both diets. It is of interest that the two groups studied were closely comparable before treatment in respect to symptoms, body weight, and size of ulcer. While ulcer symptoms disappeared more quickly from the patients that were on the bland soft ulcer diet, the patients on the nearly normal diet had healing of their ulcers more quickly and gained weight more rapidly. Patients given an intragastric milk drip had less pain than those who did not have this form of medication. Half the patients in each group received this form of therapy. A similar type of study was carried out with out-patients and here again the results were closely similar regardless of the type of diet used.

The conclusion drawn from these results is that dieting with "bland" food does not increase the rate of healing of peptic ulcers, but "these results should not be interpreted as signifying that the pathogenesis of peptic ulcers is entirely unrelated to diet. But specifically harmful or curative factors—if they exist—do not appear to have yet been adequately identified."

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